

CLAIMS

What is claimed is:

1. A method of transfecting an antigen presenting cell with at least one mRNA, comprising:
 - 5 (a) preparing a preparation essentially devoid of antisense-oriented RNA and double-stranded RNA and comprising at least one sense-oriented mRNA by:
 - 10 (i) amplifying at least one mRNA from a sample to produce a polynucleotide template, wherein the polynucleotide template comprises a promoter suitable for *in vitro* transcription operably linked only to a sense strand of the polynucleotide template; and
 - 15 (ii) *in vitro* transcribing the polynucleotide template to produce the at least one sense-oriented mRNA, wherein the polynucleotide template is not a cloned template; and
 - (b) transfecting at least one antigen presenting cell with the at least one sense-oriented mRNA from the preparation.
- 20 2. The method of claim 1, wherein the mRNA in the sample is from a cell or a virion.
3. The method of claim 2, wherein the cell is selected from the group consisting of a cancer cell and a microbial cell.
4. The method of claim 3 wherein the cancer cell is derived from a cancer selected from the group consisting of hematologic malignancies, renal cell cancer, melanoma, breast cancer, prostate cancer, testicular cancer, bladder cancer, ovarian cancer, cervical cancer, stomach cancer, esophageal cancer, pancreatic cancer, lung cancer, neuroblastoma, glioblastoma, retinoblastoma, leukemias, myelomas, lymphomas, hepatoma, adenomas, sarcomas, carcinomas, and blastomas.
- 25 30 5. The method of claim 3, wherein the microbial cell is selected from the group consisting of *Helicobacter sp.*, *Salmonella sp.*, *Shigella*

- 5 *sp.*, *Enterobacter sp.*, *Campylobacter sp.*, *Mycobacterium sp.*,
Bacillus anthracis, *Yersinia pestis*, *Francisella tularensis*, *Brucella*
sp., *Leptospira interrogans*, *Staphylococcus sp.*, *Streptococcus sp.*,
Clostridium sp., *Candida albicans*, *Plasmodium sp.*, *Leishmania sp.*,
and *Trypanosoma sp.*
- 10 6. The method of claim 2, wherein the virion is selected from the group
consisting of human immunodeficiency virus, hepatitis B virus,
hepatitis C virus, human papilloma virus, cytomegalovirus, human T-
cell lymphotropic virus, herpes simplex virus 1, herpes simplex virus
2, varicella-zoster virus, Epstein-Barr virus, influenza virus,
coronavirus, poliomyelitis virus, measles virus, mumps virus, and
rubella virus.
- 15 7. The method of claim 1, wherein the at least one sense-oriented
mRNA encodes an antigen, and wherein the antigen is translated
from the at least one sense-oriented mRNA by the at least one
transfected antigen presenting cell.
- 20 8. The method of claim 7, wherein the at least one transfected antigen
presenting cell presents the expressed antigen.
9. The method of claim 1 wherein the at least one mRNA from the
sample is a plurality of mRNAs.
- 25 10. The method of claim 9, wherein the plurality of mRNAs comprises a
total mRNA population derived from a cell or a virion.
11. The method of claim 9, wherein the plurality of mRNAs comprises a
selected fraction of a total mRNA population derived from a cell or a
virion.
- 30 12. The method of claim 11, wherein the selected fraction of the total
mRNA population is selected utilizing a subtractive hybridization
method.
13. The method of claim 11, wherein the selected fraction of the total
mRNA population is derived from a cancer cell and the selected
fraction comprises mRNAs encoding antigens unique to the cancer
cell.

14. The method of claim 1, wherein amplifying the mRNA from the sample comprises:
- 5 (a) reverse transcribing the mRNA from the sample to produce a polynucleotide template comprising a cDNA; and
- (b) amplifying the polynucleotide template cDNA using a first primer and a second primer, wherein only one of the first primer and the second primer inserts the promoter suitable for *in vitro* transcription into the polynucleotide template cDNA.
15. The method of claim 14, wherein the *in vitro* transcribing comprises *in vitro* transcribing the polynucleotide template cDNA into the sense-oriented mRNA using a polymerase specific for the promoter.
- 10 16. The method of claim 15, wherein the polymerase is a T7 polymerase.
17. The method of claim 14, wherein the first and second primers share essentially no sequence homology to one another.
- 15 18. The method of claim 14, wherein the first primer comprises a poly T stretch and a 5' sequence having essentially no sequence homology to the second primer and the second primer comprises the promoter suitable for *in vitro* transcription.
- 20 19. The method of claim 18, wherein the first primer comprises the sequence of SEQ ID NO: 2.
20. The method of claim 1, wherein transfecting is accomplished using a method selected from the group consisting of electroporation, nanoparticle-mediated transfection, peptide-mediated transfection and lipofection.
- 25 21. The method of claim 1, wherein the antigen presenting cell is selected from the group consisting of a dendritic cell and a macrophage.
22. The method of claim 21, wherein the dendritic cell is an immature dendritic cell.
- 30 23. The method of claim 21 wherein the dendritic cell is a mature dendritic cell.
24. The method of claim 1, wherein the transfecting is *in vitro*.

25. The method of claim 1, wherein the transfecting is *in situ*.
26. An mRNA loaded antigen presenting cell produced by the method of claim 1.
27. The mRNA loaded antigen presenting cell of claim 26, wherein the antigen presenting cell is a dendritic cell.
28. An mRNA loaded antigen presenting cell produced by the method of claim 2.
29. An mRNA loaded antigen presenting cell produced by the method of claim 3.
30. An mRNA loaded antigen presenting cell produced by the method of claim 4.
31. An mRNA loaded antigen presenting cell produced by the method of claim 5.
32. An mRNA loaded antigen presenting cell produced by the method of claim 6.
33. A composition comprising at least one mRNA loaded antigen presenting cell of claim 26 in a carrier.
34. A method of generating an immune response in a subject against at least one antigen, comprising introducing the mRNA loaded antigen presenting cell of claim 26 into a subject, wherein the mRNA loaded antigen presenting cell presents the at least one antigen to the immune system of the subject, thereby generating an immune response against the at least one antigen.
35. The method of claim 34, wherein the mRNA encodes at least one antigen from a cell or a virion.
36. The method of claim 35, wherein the cell is selected from the group consisting of a cancer cell and a microbial cell.
37. The method of claim 36 wherein the cancer cell is derived from a cancer selected from the group consisting of hematologic malignancies, renal cell cancer, melanoma, breast cancer, prostate cancer, testicular cancer, bladder cancer, ovarian cancer, cervical cancer, stomach cancer, esophageal cancer, pancreatic cancer, lung cancer, neuroblastoma, glioblastoma, retinoblastoma, leukemias,

myelomas, lymphomas, hepatoma, adenomas, sarcomas, carcinomas, and blastomas.

38. The method of claim 36, wherein the microbial cell is selected from the group consisting of *Helicobacter sp.*, *Salmonella sp.*, *Shigella sp.*, *Enterobacter sp.*, *Campylobacter sp.*, *Mycobacterium sp.*, *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Brucella sp.*, *Leptospira interrogans*, *Staphylococcus sp.*, *Streptococcus sp.*, *Clostridium sp.*, *Candida albicans*, *Plasmodium sp.*, *Leishmania sp.*, and *Trypanosoma sp.*
39. The method of claim 35, wherein the virion is selected from the group consisting of human immunodeficiency virus, hepatitis B virus, hepatitis C virus, human papilloma virus, cytomegalovirus, human T-cell lymphotropic virus, herpes simplex virus 1, herpes simplex virus 2, varicella-zoster virus, Epstein-Barr virus, influenza virus, coronavirus, poliomyelitis virus, measles virus, mumps virus, and rubella virus.
40. The method of claim 34 wherein the at least one mRNA from the sample is a plurality of mRNAs.
41. The method of claim 34, wherein the antigen presenting cell is selected from the group consisting of a dendritic cell and a macrophage.
42. The method of claim 41, wherein the antigen presenting cell is a dendritic cell.
43. The method of claim 42, wherein the antigen presenting cell is an immature dendritic cell.
44. The method of claim 42, wherein the antigen presenting cell is a mature dendritic cell.
45. The method of claim 44 wherein, the antigen presenting cell is an autologous antigen presenting cell obtained or derived from the subject.